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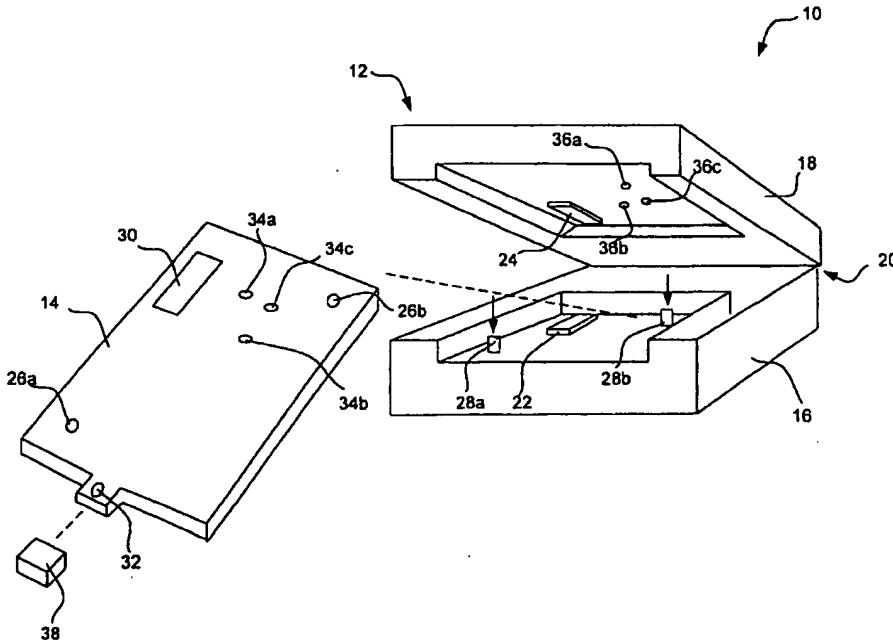
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(54) Title: PORTABLE FLOW CYTOMETER

**WO 02/10713 A2**

(57) Abstract: A portable or wearable cytometer (10) that can be used at remote locations, such as in the field or at home. The flow cytometer (10) of the present invention may help improve the healthcare of many weak, sick or elderly people by providing early detection of infection. By detecting the infection early, the infection may be more readily treatable. In military applications, the portable cytometer (10) of the present invention may help save lives by providing early detection of infection due to biological agents.



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PORTABLE FLOW CYTOMETER

Cross-Reference to Related Co-Pending Applications

This Application is related to co-pending U.S. Patent Application Serial No.

5 _____ to Cabuz et al., filed _____, and entitled "FLUID DRIVING SYSTEM FOR FLOW CYTOMETRY", U.S. Patent Application Serial No. _____ to Cabuz et al., filed _____, and entitled "OPTICAL DETECTION SYSTEM FOR FLOW CYTOMETRY", and U.S. Patent Application Serial No. 09/404,560, filed September 23, 1999, and entitled "ADDRESSABLE 10 VALVE ARRAYS FOR PROPORTIONAL PRESSURE OR FLOW CONTROL", all of which are incorporated herein by reference.

Field of the Invention

The present invention relates generally to flow cytometers. More particularly, the present invention relates to portable flow cytometers that sense optical properties 15 of microscopic biological particles in a flow stream.

Background of the Invention

Flow cytometry is a technique that is used to determine certain physical and chemical properties of microscopic biological particles by sensing certain optical properties of the particles. To do so, the particles are arranged in single file using 20 hydrodynamic focussing within a sheath fluid. The particles are then individually interrogated by a light beam. Each particle scatters the light beam and produces a scatter profile. The scatter profile is often identified by measuring the light intensity at different scatter angles. Certain physical and/or chemical properties of each particle can then be determined from the scatter profile.

25 Flow cytometry is currently used in a wide variety of applications including hematology, immunology, genetics, food science, pharmacology, microbiology, parasitology and oncology, to name a few. A limitation of many commercially available flow cytometer systems is that they are relatively large bench top instruments that must remain in a central laboratory environment. Accordingly, the 30 use of such flow cytometers is often not available in remote locations or for continuous hematological monitoring.

Summary of the Invention

The present invention overcomes many of the disadvantages of the prior art by providing a portable or wearable cytometer that can be used at remote locations, such as at home or in the field. Such a flow cytometer may help improve healthcare of 5 patients by providing detailed individual hematological evaluation and uncovering statistical trends. By detecting an infection early, the infection may be more readily treatable.

In military applications, the portable cytometer of the present invention may help save lives by providing early detection of infection due to biological agents. It is 10 known that expanded activity in the biological sciences has increased the probability of accidental exposure to dangerous biological agents. The ease of manufacturing such agents also raises a serious threat to their use by terrorists, regional powers or developing third world nations. The lack of safeguards in international agreements outlawing biological warfare, and compelling evidence that those agreements may 15 have been violated, reinforces the need for a strong capability for biological defense. During Desert Storm, American forces were not prepared to operate in a biological environment. Pre-exposure detection of pathogen agents, as well as post-exposure detection of incipient infections had to be used cooperatively to ensure efficient protection during biological warfare.

20 As part of the body's natural defense against antigens, the white blood cell count increases at the onset of infection. There are several types of white blood cells including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Lymphocytes create antibodies that attack the invaders and mark them for destruction by the neutrophils and macrophages. In an individual without chronic diseases (such 25 as tuberculosis or cancer), an increase in the percentage of lymphocytes in the overall white cell count is an indication of a viral infection. On the other side, an increase in the percentage of the neutrophils is an indication of a developing bacterial infection. Through counting of neutrophils and lymphocytes, a clear infection warning can be issued with differentiation between viral or bacterial causes.

30 The first clinical symptoms of infection from some bacterial agents such as bacillus anthrax appear after one to six days. In 99% of the cases, patients showing symptoms from anthrax cannot be treated, and will most likely die. However, if treatment is given before the first symptoms appear, most patients can be successfully

treated. Accordingly, it would be highly desirable to provide an early alert and potential therapeutic intervention for hematologic abnormalities before symptoms occur. In many cases, such an early alert and treatment may greatly improve the outcome for many patients.

5 In one illustrative embodiment of the present invention, a portable cytometer is provided for identifying and/or counting selected particles in a fluid sample such as a blood sample. One illustrative portable cytometer includes a fluid receiver for receiving the fluid sample. One or more reservoirs are provided for storing supporting fluids such as lyse and sheath fluids. For many commercial flow cytometer systems, a
10 precision fluid driving system is used for providing precise pressures to the fluids. A limitation of this approach is that precision fluid driving systems can be bulky, complex and may require significant power.

15 To avoid many of these limitations, one illustrative embodiment uses a non-precision fluid driver that is controlled by a closed loop feedback path. The non-precision fluid driver is coupled to the fluid receiver and the various supporting fluid reservoirs, and applies separate pressures to the sample fluid and the supporting fluids.
20 To control the velocity of the sample fluid and the supporting fluids, one or more valves are coupled to the fluid driver. The valves are used to regulate the non-precision pressures that are applied to the sample fluid and the supporting fluids by the non-precision fluid driver.

25 To complete the feedback loop, flow sensors are provided downstream of the fluid driver to measure the fluid velocity of the sample fluid and the supporting fluids. A controller or processor receives the signals from the flow sensors, and adjusts the appropriate valves so that the desired fluid velocities of the sample fluid and supporting fluids are achieved. The flow sensors are preferably thermal anemometer type flow sensors.

30 In one embodiment, the non-precision fluid driver is manually powered. A manually powered fluid driver may include, for example, a bulb with check valve or a plunger. In either case, the manually generated pressure is preferably provided to a first pressure chamber. A first valve is then provided for controllably releasing the pressure in the first pressure chamber to a second pressure chamber. A second valve may be provided in the second pressure chamber for controllably venting the pressure in the second pressure chamber. The controller opens the first valve when the fluid

flow in the downstream fluid stream drops below a first predetermined value and opens the second valve when the fluid flow in the downstream fluid stream increases above a second predetermined value. Each valve is preferably an array of electrostatically actuated microvalves that are individually addressable and

5 controllable.

The controlled sample fluid and supporting fluids are provided to a fluidic circuit. The fluidic circuit performs hydrodynamic focusing, which causes the desired particles to fall into single file along a core stream surrounded by a sheath fluid. One or more light sources provide light through the flow stream, and one or more light

10 detectors detect the scatter profile of the particles in the flow stream. A processing block uses the output signals from the light detectors to identify and/or count selected particles in the core stream.

The portable cytometer may be provided in a housing sufficiently small to be "wearable". In one embodiment, the housing is sized similar to a wrist watch. The

15 wearable housing may include, for example, a base, a cover, and a hinge that secures the base to the cover. The non-precision fluid driver and regulating valves may be incorporated into the cover, while the fluid reservoirs, flow sensors and fluidic circuit may be incorporated into a removable cartridge that is inserted into the housing. Preferably, the fluidic circuit dilutes the blood sample, performs red cell lysing, and

20 performs hydrodynamic focusing for flow and core stream formation. The light sources are preferably situated in either the base or the cover, and aligned with the flow stream of the removable cartridge. The light detectors are preferably provided generally opposite the light sources. The processor and batteries may be provided in either the base or the cover of the housing.

25 The light sources may include a linear array of first light sources along a first light source axis. The first light source axis is preferably rotated relative to the central axis of the flow stream. A lens may be provided adjacent each light source to focus the light at the particles in the core stream. A first set of light detectors may then be placed in-line with each of the light source. Such an arrangement can be used to

30 determine, for example, the alignment and width of the core stream within the flow stream. If the core stream of particles is not in proper alignment, the controller can adjust the fluid velocity of the sample fluid or one of the supporting fluids to bring the

core stream into alignment. The first set of light detectors may also be used to detect the velocity and size of each particle, as well as the number of particles.

A second set of the light sources may be provided along a second light source axis. A lens may be provided adjacent each light source to focus the light at the 5 particles in the core stream. A second set of light detectors may then be placed on either side of the in-line position of each light source for measuring the small angle scattering (SALS) produced by selected particles in the flow stream.

The second set of light sources may also be used in conjunction with the first set of light sources to determine the time-of-flight or velocity of the particles in the 10 flow stream. By knowing the velocity of the particles, small variations in the flow rate caused by the fluid driver can be minimized or removed by the controller.

A third set of light sources may be provided along a third light source axis. A lens may be provided adjacent each light source to provide collimated light to the flow stream. Annular light detectors are then preferably placed opposite the light sources 15 for measuring the forward angle scattering (FALS) produced by the selected particles in the flow stream. Each of the first, second and third set of light sources preferably include an array of lasers such as Vertical Cavity Surface Emitting Lasers (VCSEL) fabricated on a common substrate. Each of the first, second and third set of light detectors preferably include an array of photo detectors such as p-i-n photodiodes, 20 GaAs photodiodes with integrated FET circuits, Resonant Cavity Photo Detectors (RCPD), or any other suitable light detector.

The selected particles are preferably neutrophils and/or lymphocytes white blood cells. By examining the scatter profile of each particle, the portable cytometer of the present invention preferably identifies and counts the neutrophils and 25 lymphocytes in a blood sample, and provide a clear infection warning with differentiation between viral and bacterial causes.

Brief Description of the Drawings

Other objects of the present invention and many of the attendant advantages of the present invention will be readily appreciated as the same becomes better 30 understood by reference to the following detailed description when considered in connection with the accompanying drawings, in which like reference numerals designate like parts throughout the figures thereof and wherein:

Figure 1 is a perspective view of an illustrative portable cytometer in accordance with the present invention;

Figure 2 is a schematic view of the illustrative portable cytometer of Figure 1;

5 Figure 3 is a more detailed schematic diagram showing the portable cytometer of Figure 2 with the cover not yet depressed;

Figure 4 is a more detailed schematic diagram showing the portable cytometer of Figure 2 with the cover depressed;

Figure 5 is a schematic diagram showing an illustrative manual fluid driver having a bulb and check valve;

10 Figure 6 is a graph showing proportional pressure control of an addressable array of microvalves;

Figure 7 is a schematic diagram showing the formation of a flow stream by the hydrodynamic focusing block 88 of Figure 3;

15 Figure 8 is a schematic diagram showing an array of light sources and an array of light detectors for analysis of the core stream 160 of Figure 7.

Figure 9 is a graph showing the light intensity produced along the light source axis of Figure 8;

Figure 10 is a schematic diagram showing an illustrative light source and detector pair of Figure 8;

20 Figure 11 is a schematic diagram showing three separate arrays of light sources and detectors, each positioned along a different light source axis that is slightly rotated relative to the central flow axis of the flow stream of Figure 7;

Figure 12 is a schematic diagram showing an illustrative light source and detector pair of the first array shown in Figure 11;

25 Figure 13 is a schematic diagram showing an illustrative light source and detector pair of the second array shown in Figure 11;

Figure 14 is a schematic diagram showing an illustrative light source and detector pair of the third array shown in Figure 11; and

30 Figure 15 is a perspective view of an illustrative embodiment of the portable cytometer of the present invention adapted to be worn around the wrist.

Detailed Description of the Preferred Embodiments

Figure 1 is a perspective view of an illustrative portable cytometer in accordance with the present invention. The portable cytometer is generally shown at

10, and includes a housing 12 and a removable or replaceable cartridge 14. The illustrative housing 12 includes a base 16, a cover 18, and a hinge 20 that attaches the base 16 to the cover 18. The base 16 includes an array of light sources 22, associated optics and the necessary electronics for operation of the cytometer. The cover 12
5 includes a manual pressurizing element, pressure-chambers with control microvalves, and an array of light detectors 24 with associated optics.

The removable cartridge 14 preferably receives a sample fluid via a sample collector port 32. A cap 38 may be used to protect the sample collector port 32 when the removable cartridge 14 is not in use. The removable cartridge 14 preferably performs blood dilution, red cell lysing, and hydrodynamic focusing for core formation. The removable cartridge 14 may be constructed similar to the fluidic circuits available from Micronics Technologies, some of which are fabricated using a laminated structure with etched channels.
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The removable cartridge 14 is inserted into the housing when the cover 18 is in the open position. The removable cartridge 14 may include holes 26a and 26b for receiving registration pins 28a and 28b in the base 16, which help provide alignment and coupling between the different parts of the instrument. The removable cartridge 14 also preferably includes a transparent flow stream window 30, which is in alignment with the array of the light sources 22 and light detectors 24. When the cover is moved to the closed position, and the system is pressurized, the cover 18 provides controlled pressures to pressure receiving ports 34a, 34b, and 34c in the removable cartridge 14 via pressure providing ports 36a, 36b and 36c, respectively.
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To initiate a test, the cover 18 is lifted and a new cartridge 14 is placed and registered onto the base 16. A blood sample is introduced into the sample collector 32. The cover 18 is closed and the system is manually pressurized. Once pressurized, the instrument performs a white blood cell cytometry measurement. The removable cartridge 14 provides blood dilution, red cell lysing, and hydrodynamic focusing for core formation. The light sources 22, light detectors 24 and associated control and processing electronics perform differentiation and counting of white blood cells based on light scattering signals. Rather than using a hinged construction for the housing 12, it is contemplated that a sliding cartridge slot or any other suitable construction may be used.
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Figure 2 is a schematic view of the illustrative portable cytometer of Figure 1. As above, the base 16 may include an array of light sources 22, associated optics and the necessary control and processing electronics 40 for operation of the cytometer. The base 16 may also include a battery 42 for powering the cytometer. The cover 12 is shown having a manual pressurizing element 44, pressure-chambers 46a, 46b and 46c with control microvalves, and an array of light detectors 24 with associated optics.

The removable cartridge 14 may receive a sample fluid via the sample collector port 32. When pressurized by the cover 18, the removable cartridge 14 performs blood dilution, red cell lysing, and hydrodynamic focusing for core formation in a preferred embodiment. Once formed, the core is provided down a flow stream path 50, which passes the flow stream window 30 of Figure 1. The array of light sources 22 and associated optics in the base provide light through the core stream via the flow stream window 30. The array of light detectors and associated optics receive scattered and non-scattered light from the core, also via the flow stream window 30. The controller or processor 40 receives output signals from the array of detectors, and differentiates and counts selected white blood cells that are present in the core stream.

It is contemplated that the removable cartridge 14 may include a fluid control block 48 for helping control the velocity of each of the fluids. In the illustrative embodiment, the fluid control block 48 includes flow sensors for sensing the velocity of the various fluids and report the velocities to the controller or processor 40. The controller or processor 40 may then adjust the microvalves associated with pressure-chambers 46a, 46b and 46c to achieve the desired pressures and thus desired fluid velocities for proper operation of the cytometer.

Because blood and other biological waste can spread disease, the removable cartridge 14 preferably has a waste reservoir 52 downstream of the flow stream window 30. The waste reservoir 52 receives and stores the fluid of the flow stream in the removable cartridge 14. When a test is completed, the removable cartridge may be removed and disposed of, preferably in a container compatible with biological waste.

Figure 3 is a more detailed schematic diagram showing the portable cytometer of Figure 2 with the cover 18 not yet depressed. Figure 4 is a more detailed schematic diagram showing the portable cytometer of Figure 2 with the cover depressed. The

cover 18 is shown having a manual pressurizing element 44, pressure-chambers 46a, 46b and 46c, and control microvalves generally shown at 60. The array of light sources and detectors are not shown in these Figures.

There are three pressure chambers 46a, 46b and 46c, one for each fluid to be
5 pressurized. In the illustrative embodiment, pressure chamber 46a provides pressure to a blood sample reservoir 62, pressure chamber 46b provides pressure to a lyse reservoir 64, and pressure chamber 46c provides pressure to a sheath reservoir 66. The size and shape of each pressure chamber 46a, 46b and 46c may be tailored to provide the desired pressure characteristics to the corresponding fluid.

10 Pressure chamber 46a includes a first pressure chamber 70 and a second pressure chamber 72. A first valve 74 is provided between the first pressure chamber 70 and the second pressure chamber 72 for controllably releasing the pressure in the first pressure chamber 70 to a second pressure chamber 72. A second valve 76, in fluid communication with the second pressure chamber 72, controllably vents the
15 pressure in the second pressure chamber 72. Each valve is preferably an array of electrostatically actuated microvalves that are individually addressable and controllable, as described in, for example, co-pending U.S. Patent Application Serial Number 09/404,560, entitled "ADDRESSABLE VALVE ARRAYS FOR PROPORTIONAL PRESSURE OR FLOW CONTROL", and incorporated herein by reference. Pressure chambers 46b and 46c include similar valves to control the pressures applied to the lyse reservoir 64 and sheath reservoir 66, respectively. Alternatively, each valve may be an array of electrostatically actuated microvalves that are pulse modulated with a controllable duty cycle to achieve a controlled "effective"
20 flow or leak rate.

25 The removable cartridge 14 has pressure receiving ports 34a, 34b, and 34c for receiving the controlled pressures from the cover 18. The controlled pressures are provided to the blood reservoir 62, lyse reservoir 64 and sheath reservoir 66, as shown. The lyse reservoir 64 and sheath reservoir 66 are preferably filled before the removable cartridge 14 is shipped for use, while the blood reservoir 62 is filled from
30 sample collector port 32. A blood sample may be provided to the sample collector port 32, and through capillary action, the blood sample is sucked into the blood reservoir 62. Once the blood sample is in the blood reservoir 62, the cover 18 may be closed and the system may be pressurized.

A flow sensor is provided in-line with each fluid prior to hydrodynamic focussing. Each flow sensor 80, 100 and 102 measures the velocity of the corresponding fluid. The flow sensors are preferably thermal anemometer type flow sensors, and more preferably microbridge type flow sensor. Microbridge flow sensors 5 are described in, for example, U.S. Patent No. 4,478,076, U.S. Patent No. 4,478,077, U.S. Patent No. 4,501,144, U.S. Patent No. 4,651,564, U.S. Patent No. 4,683,159, and U.S. Patent No. 5,050429, all of which are incorporated herein by reference. An output signal from each flow sensor 80, 100 and 102 is provided to controller or processor 40.

10 The controller or processor 40 opens the first valve 74 when the velocity of the blood sample drops below a first predetermined value and opens the second valve 76 when the velocity of the blood sample increases above a second predetermined value. Valves 84, 86, 94 and 96 operate in a similar manner to control the velocities of the lyse and sheath fluids.

15 During operation, and to pressurize the system, the manual pressurizing element 44 is depressed. In the example shown, the manual pressurizing element 44 includes three plungers, with each plunger received within a corresponding one of the first pressure chambers. The plungers create a relatively high non-precision pressure in the first pressure chambers. Lower, controlled pressures are built in the secondary 20 chambers by opening the first valves 70, 84 and 94, which produce a controllable leak into the secondary chambers. If too much pressure builds up in the secondary pressure chambers, the corresponding vent valve 76, 86 and 96 are opened to relieve the pressure.

When closing the cover 18, the normally open first valves 74, 84 and 94 are 25 closed while the vent valves 76, 86 and 96 are open. When a predetermined pressure P is achieved in the first pressure chambers, the vent valves 76, 86 and 96 are closed, and the first valves 74, 84 and 94 are opened to build a lower pressure P' in the secondary pressure chambers. The controlled pressure in the secondary pressure chambers provide the necessary pressures to the fluidic circuit of the removable 30 cartridge 14 to produce fluid flow for the blood, lyse and sheath. The velocity of the fluid flow is then measured by the downstream flow sensors 80, 100 and 102. Each flow sensor provides an output signal that is used by the controller or processor 40 to

control the operation of the corresponding first valve and vent valve to provide a desired and constant flow rate for each fluid.

Downstream valves generally shown at 110 may also be provided. Controller or processor 40 may close downstream valves 110 until the system is pressurized.

5 This may help prevent the blood, lyse and sheath from flowing into the fluid circuit before the circuit is pressurized. In another embodiment, downstream valves 110 are opened by mechanical action when the cover is closed.

Figure 5 is a schematic diagram showing an illustrative manual fluid driver having a bulb 100 and check valve 102. The check valve 102 is preferably a one way valve that allows air in but not out of the first pressure chamber 104. When the bulb 100 is depressed, the air in the interior 106 of the bulb 100 is forced through the check valve 102 and into the first pressure chamber 104. Preferably, another a one-way vent valve 105 is provided that allows air in from the atmosphere but not out of the interior 106 of the bulb 100. Thus, when the bulb is released, the one-way vent valve 105 may allow replacement air to flow into bulb 100.

Rather than using a manually operated fluid driver, it is contemplated that any relatively small pressure source may be used including, for example, an electrostatically actuated meso-pump. One such meso-pump is described in, for example, U.S. Patent No. 5,836,750 to Cabuz, which is incorporated herein by reference.

Figure 6 is a graph showing proportional pressure control produced by a 8x7 addressable array of microvalves. To create the graph shown in Figure 7, 6.5 psi was applied to a first pressure chamber 120. A small opening was provided to a second pressure chamber 122. The microvalves are shown at 124, and vent the pressure in the second pressure chamber 122. By changing the number of addressable microvalves that are closed, the pressure in the second pressure chamber can be changed and controlled. In the graph shown, the pressure in the second pressure chamber 122 could be changed from about 0.6 psi, when zero of the 8x7 array of microvalves closed, to about 6.5 psi, when all of the 8x7 array of microvalves are closed. These low power, micromachined silicon microvalves can be used for controlling pressures up to 10psi and beyond.

Figure 7 is a schematic diagram showing the formation of a flow stream and core by the hydrodynamic focusing block 88 of Figure 3. The hydrodynamic focusing

block 88 receives blood, lyse and sheath at controlled velocities from the fluid driver. The blood is mixed with the lyse, causing the red blood cells to be removed. This is often referred to as red cell lysing. The remaining white blood cells are provided down a central lumen 150, which is surrounded by sheath fluid to produce a flow stream 50. The flow stream 50 includes a core stream 160 surrounded by the sheath fluid 152. The dimensions of the channel are reduced as shown so that the white blood cells 154 and 156 are in single file. The velocity of the sheath fluid is preferably about 9 times that of the core stream 160. However, the velocity of the sheath fluid and core stream 160 remain sufficiently low to maintain laminar flow in the flow channel.

Light emitters 22 and associated optics are preferably provided adjacent one side of the flow stream 50. Light detectors 24 and associated optics are provided on another side of the flow stream 50 for receiving the light from the light emitters 22 via the flow stream 50. The output signals from the light detectors 24 are provided to controller or processor 40, wherein they are analyzed to identify and/or count selected white blood cells in the core stream 160.

Figure 8 is a schematic diagram showing an array of light sources and an array of light detectors for analysis of the core stream 160 of Figure 7. The light sources are shown as "+" signs and the detectors are shown at boxes. In the embodiment shown, the array of light sources is provided adjacent one side of the flow stream 50, and the array of light detectors is provided adjacent the opposite side of the flow stream. Each of the light detectors is preferably aligned with a corresponding one of the light sources. The array of light sources and the array of light detectors are shown arranged along a light source axis 200 that is slightly rotated relative to the axis 202 of the flow stream 50.

The array of light sources is preferably an array of lasers such as Vertical Cavity Surface Emitting Lasers (VCSEL) fabricated on a common substrate. Because of their vertical emission, VCSELs are ideally suited for packaging in compact instruments such as a portable cytometer. Preferably, the VCSELs are "red" VCSELs that operate at wavelengths that are less than the conventional 850 nm, and more preferably in the 670 nm to 780 nm range. Red VCSELs may have a wavelength, power and polarization characteristic that is ideally suited for scatter measurements.

Some prior art cytometer bench models use a single 9mW edge-emitting laser with a wavelength of 650 nm. The beam is focussed to a 10x100 micron elongated shape to cover the uncertainty in particle position due to misalignment and width of the core stream. In contrast, the output power of the red VCSELs of the present invention, operating at 670 nm, is typically around 1 mW for a 10x10 micron emitter and 100-micron spacing. Thus, the total intensity of the light from a linear array of ten red VCSELs may be essentially the same as that of some prior art bench models.

Using a linear array of lasers oriented at an angle with respect to the flow axis 202 offers a number of important advantages over the single light source configuration 10 of the prior art. For example, a linear array of lasers may be used to determining the lateral alignment of the path of the particles in the core steam. One source of uncertainty in the alignment of the particle stream is the width of the core flow, which leads to statistical fluctuations in the particle path position. These fluctuations can be determined from analysis of the detector data and can be used by the controller or 15 processor 40 to adjust the valves of the fluid driver in order to change the relative pressures that are applied to the sample fluid and the supporting fluids to change the alignment of the selected particles in the flow stream.

To determine the lateral alignment of the cells in the fluid stream 50, the cells pass through several focussed spots produced by the linear array of VCSELs. The 20 cells produce a drop in signal in the corresponding in-line reference detectors. The relative strengths of the signals are used by the controller or processor 40 to determine the center of the particle path and a measure of the particle width.

For determining particle path and size, the lasers are preferably focussed to a series of Gaussian spots (intensity on the order of 1000W/cm^2) in the plane of the 25 core flow. The spots are preferably about the same size as a white blood cell (10-12 um). Illustrative Gaussian spots are shown in Figure 9. Arrays of detectors and their focussing optics are provided on the opposite side of the fluid stream. Lenses with fairly large F-numbers are used to provide a working space of several hundred microns for the cytometer section of the removable cartridge.

Another advantage of using a linear array of lasers rather than a single laser 30 configuration is that the velocity of each cell may be determined. Particle velocity can be an important parameter in estimating the particle size from light scatter signals. In conventional cytometry, the particle velocity is extrapolated from the pump flow rates.

A limitation of this approach is that the pumps must be very precise, the tolerance of the cytometer flow chambers must be tightly controlled, no fluid failures such as leaks can occur, and no obstructions such as microbubbles can be introduced to disturb the flow or core formation.

5 To determine the velocity of each cell, the system may measure the time required for each cell to pass between two adjacent or successive spots. For example, and with reference to Figure 8, a cell may pass detector 208 and then detector 210. By measuring the time required for the cell to travel from detector 208 to detector 210, and by knowing the distance from detector 208 to detector 210, the controller or
10 processor 40 can calculate the velocity of the cell. This would be an approximate velocity measurement. This is often referred to as a time-of-flight measurement. Once the velocity is known, the time of travel through the spot on which the particle is centered (a few microseconds) may provide a measure of particle length and size.

It is contemplated that the particle velocity can also be used to help control the fluid driver. To reduce the size, cost and complexity of the present invention, the replaceable cartridge of Figure 1 may be manufactured from a plastic laminate or molded parts. While such manufacturing techniques may provide inexpensive parts, they are typically less dimensionally precise and repeatable, with asymmetrical dimensions and wider tolerance cross-sections. These wider tolerances may produce variations in particle
15 velocity, particularly from cartridge to cartridge. To help compensate for these wider tolerances, the time-of-flight measurement discussed above can be used by the controller or processor 40 to adjust the controlled pressures applied to the blood, lysis and sheath fluid streams such that the particles in the core stream have a relatively constant velocity.
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To further evaluate the cell size, it is contemplated that laser beams may be focused both along the cell path and across the cell path. Additionally, multiple samples across the cell may be analyzed for texture features, to correlate morphological features to other cell types. This may provide multiple parameters about cell size that may help separate cell types from one another.
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Another advantage of using a linear array of lasers rather than a single layer configuration is that a relatively constant light illumination may be provided across the flow channel. This is accomplished by overlapping the Gaussian beams from adjacent VCSELs, as shown in Figure 9. In prior art single laser systems, the light illumination across the flow channel typically varies across the channel. Thus, if a
30

particle is not in the center of the flow channel, the accuracy of subsequent measurements may be diminished.

To perform the above described measurements, each detector in Figure 8 may be a single in-line detector. To measure FALS and SALS scatter, however, each 5 detector may further include two annular detectors disposed around the in-line detector, as shown in Figure 10. Referring to Figure 10, a VCSEL 218 is shown providing light in an upward direction. The light is provided through a lens 220, which focuses the light to a Gaussian spot in the plane of the core flow. lens 220 may be a microlens or the like, which is either separate from or integrated with the VCSEL 10 218. The light passes through the core flow, and is received by another lens 222, such as a diffractive optical element. Lens 222 provides the light to in-line detector 226 and annular detectors 228 and 230. The in-line detector 226 detects the light that is not significantly scattered by the particles in the core stream. Annular detector 228 detects the forward scatter (FALS) light, and annular detector 230 detects the small 15 angle scatter (SALS) light.

Figure 11 shows another illustrative embodiment of the present invention that includes three separate arrays of light sources and light detectors. Each array of light sources and light detectors are positioned along a different light source axis that is slightly rotated relative to the central flow axis of the flow stream. By using three 20 arrays, the optics associated with each array may be optimized for a particular application or function. For detecting small angle scattering (SALS), laser light that is well-focussed on the plane of the core flow is desirable. For detecting forward scattering (FALS), collimated light is desirable.

Referring specifically to Figure 11, a first array of light sources and light 25 detectors is shown at 300. The light sources and light detectors are arranged in a linear array along a first light source axis. The first light source axis is rotated relative to the flow axis of the flow stream. The light sources and light detectors may be similar to that described above with respect to Figure 8, and preferably are used to measure, for example, the lateral alignment of the cells in the flow stream, the particle 30 size, and the velocity of the particles.

Figure 12 is a schematic diagram showing an illustrative light source and detector pair of the first array 300 shown in Figure 11. A VCSEL 302 is shown providing light in an upward direction. The light is provided through a lens 304,

which focuses the light to a Gaussian spot in the plane of the core flow. The light passes through the core flow, and is received by another lens 306. Lens 306 provides the light to in-line detector 308. The in-line detector 308 detects the light that is not significantly scattered by the particles in the core stream.

5 A second array of light sources and light detectors is shown at 310. The light sources are arranged in a linear array along a second light source axis that is rotated relative to the flow axis of the flow stream. The light detectors include three linear arrays of light detectors. One array of light detectors is positioned in line with the linear array of light sources. The other two linear arrays of light detectors are placed
10 on either side of the in-line array of light detectors, and are used for measuring the small angle scattering (SALS) produced by selected particles in the flow stream.

Figure 13 is a schematic diagram showing an illustrative light source and corresponding detectors of the second array shown in Figure 11. A VCSEL 320 is shown providing light in an upward direction. The light is provided through a lens
15 322, which focuses the light to a Gaussian spot in the plane of the core flow. The light passes through the core flow, and is received by another lens 324, such as a diffractive optical element (DOE) 324. Lens 324 provides the light to the in-line detector 326 and the two corresponding light detectors 328 and 330 placed on either side of the in-line light detector 326.

20 The in-line detector 326 may be used to detect the light that is not significantly scattered by the particles in the core stream. Thus, the in-line linear array of light detectors of the second array 302 may be used to provide the same measurements as the in-line array of detectors of the first array 300. The measurements of both in-line arrays of detectors may be compared or combined to provide a more accurate result.
25 Alternatively, or in addition, the in-line detectors of the second array 302 may be used as a redundant set of detectors to improve the reliability of the cytometer.

It is contemplated that the in-line detectors of the second array 302 may also be used in conjunction with the in-line detectors of the first array 300 to more accurately determine the time-of-flight or velocity of the particles in the flow stream.
30 The measurement may be more accurate because the distance between detectors may be greater. As indicated above, by knowing the velocity of the particles, small variations in the flow rate caused by the fluid driver can be minimized or removed by the controller.

Light detectors 328 and 330 of Figure 13 are used to measure the small angle scattering (SALS) produced by selected particles in the flow stream. The light detectors 328 and 330 are therefore preferably spaced sufficiently from the in-line detector 326 to intercept the small angle scattering (SALS) produced by selected particles in the flow stream.

Referring back to Figure 11, a third array of light sources and light detectors 350 is preferably provided to measure the forward angle scattering (FALS) produced by selected particles in the flow stream. The light sources are arranged in a linear array along a third light source axis that is rotated relative to the flow axis of the flow stream. Each light source preferably has a corresponding light detector, and each light detector is preferably annular shaped with a non-sensitive region or a separate in-line detector in the middle. The annular shaped light detectors are preferably sized to intercept and detect the forward angle scattering (FALS) produced by selected particles in the flow stream.

Figure 14 is a schematic diagram showing an illustrative light source and detector pair of the third array of light sources and light detectors 350 shown in Figure 11. A VCSEL 360 is shown providing light in an upward direction. The light is provided through a lens 362 such as a collimating lens, which provides substantially collimated light to the core flow. As indicated above, collimated light is desirable for detecting forward scattering (FALS) light. The light passes through the core flow, and is received by another lens 364. Lens 364 provides the received light to the annular shaped detector 368.

The annular shaped detector 378 is preferably sized to intercept and detect the forward angle scattering (FALS) produced by selected particles in the flow stream. A non-sensitive region or a separate in-line detector 370 may be provided in the middle of the annular shaped detector 368. If a separate in-line detector 370 is provided, it can be used to provide the same measurement as the in-line detectors of the first array 300 and/or second array 302. When so provided, the measurements from all three in-line arrays of detectors of first array 300, second array 302 and third array 350 may be compared or combined to provide an even more accurate result. The in-line detectors of the third array 302 may also be used as another level or redundancy to improve the reliability of the cytometer.

It is contemplated that the in-line detectors of the third array 350 may also be used in conjunction with the in-line detectors if the first array 300 and/or second array 302 to more accurately determine the time-of-flight or velocity of the particles in the flow stream. The measurement may be more accurate because the distance between 5 detectors may be greater. As indicated above, by knowing the velocity of the particles, small variations in the flow rate caused by the fluid driver can be minimized or removed by the controller.

By using three separate arrays of light sources and detectors, the optics associated with each array can be optimized for the desired application. As can be 10 seen, the optics associated with the first array 300 are designed to provide well-focussed laser light on the plane of the core flow. This helps provide resolution to the alignment, size and particle velocity measurements performed by the first array 300. Likewise, the optics associated with the second array 302 are designed to provide well-focussed laser light on the plane of the core flow. Well focussed light is 15 desirable when measuring the small angle scattering (SALS) produced by selected particles in the flow stream. Finally, the optics associated with the third array 350 are designed to provide collimated light to the core flow. As indicated above, collimated light is desirable when measuring forward angle scattering (FALS) produced by selected particles in the flow stream.

20 Figure 15 is a perspective view of an illustrative embodiment of the portable cytometer of the present invention adapted to be worn around the wrist. The portable cytometer is shown at 400, and may be similar to that shown in Figure 1. A band 402 secures the portable cytometer 400 to the wrist of a user.

As indicated above, the user may obtain a removable cartridge and provide a 25 blood sample to the sample collector port 32 (see Figure 1) of the removable cartridge. The blood sample may be collected by, for example, a finger prick. The user may then insert the removable cartridge into the housing, and manually pressurize the system. The portable cytometer may then provide a reading that indicates if the user should seek medical treatment. The reading may be a visual reading, an audible sound 30 or any other suitable indicator.

Rather than obtaining the blood sample by a finger prick or the like, it is contemplated that a catheter 404 or the like may be inserted into a vein of the user and attached to the sample collector port 32. This may allow the system to automatically

collect a blood sample from the user whenever a reading is desired. Alternatively, it is contemplated that the portable cytometer may be implanted in the user, with the sample collector port 32 connected to a suitable blood supply.

Having thus described the preferred embodiments of the present invention,
5 those of skill in the art will readily appreciate that the teachings found herein may be applied to yet other embodiments within the scope of the claims hereto attached.

WHAT IS CLAIMED IS:

1. A portable cytometer (10) for identifying and/or counting selected particles in a sample fluid, comprising:
 - a fluid receiver (32) for receiving the sample fluid;
 - at least one reservoir (64) for storing one or more supporting fluids;
 - a non-precision fluid driver coupled to the fluid receiver (32) and the at least one reservoir (64) for applying separate pressures to the sample fluid and the one or more supporting fluids to provide a fluid velocity to each of the sample fluid and the one or more supporting fluids;
 - valve means (74) coupled to the fluid driver for regulating the separate pressures that are applied to the sample fluid and the one or more supporting fluids;
 - at least one flow sensor (80) for measuring the fluid velocity of the sample fluid and the one or more supporting fluids provided by the fluid driver;
 - control means (40) coupled to the valve means (74) and the at least one flow sensor (80) for controlling the valve means (74) so that the fluid velocities of the sample fluid and each of the one or more supporting fluids are at desired levels;
 - a fluidic circuit (88) for receiving the sample fluid and the one or more supporting fluids from the fluid driver, and for forming a flow stream (50);
 - light providing means (22) for providing a light through the flow stream (50);
 - light receiving means (24) for receiving the light from the flow stream (50), and for providing at least one signal in response thereto; and
 - processing means (40) for receiving the at least one signal from the light receiving means (24) and for identifying and/or counting selected particles therein.
2. A portable cytometer (10) according to claim 1, wherein the non-precision fluid driver is manually powered.
3. A portable cytometer (10) according to claim 2, wherein the non-precision fluid driver includes at least one plunger received within a pressure chamber (70), wherein the plunger is manually depressed into the pressure chamber (70).

4. A portable cytometer (10) according to claim 1, wherein the non-precision fluid driver includes an electrostatic meso-pump.
5. A portable cytometer (10) according to claim 1, wherein the processing means (40) identifies the velocity of selected particles in the flow stream (50).
6. A portable cytometer (10) according to claim 1, wherein the processing means (40) identifies the size of selected particles in the flow stream (50).
7. A portable cytometer (10) according to claim 1, wherein the processing means (40) identifies the alignment of selected particles in the flow stream (50).
8. A portable cytometer (10) according to claim 7, wherein the processing means (40) provides one or more signals that are used for adjusting the valve means (72) in order to change the relative pressures that are applied to the sample fluid and the one or more supporting fluids to change the alignment of the selected particles in the flow stream (50).
9. A wearable cytometer for analyzing selected particles in a sample fluid, comprising:
 - a removable cartridge (14) for receiving the sample fluid and for preparing the sample fluid for analysis including performing hydrodynamic focusing to form a flow stream (50) having a core stream (160) of particles, the cartridge (14) having one or more pressure receiving ports (34) for receiving one or more controlled pressures;
 - a housing (12) adapted to receive the removable cartridge (14), said housing (12) having:
 - pressure applying means for applying a controlled pressure to the one or more pressure receiving ports (34) of the removable cartridge (14);
 - one or more light providing means (22) positioned adjacent the flow stream of the removable cartridge (14) for providing light through the flow stream (50);

one or more light receiving means (24) provided adjacent the flow stream of the removable cartridge (14) for receiving the light from the flow stream (50), and for providing at least one signal in response thereto; and

processing means (40) for receiving the at least one signal from the light receiving means (24) and for analyzing the at least one signal to determine predetermined information relative to the selected particles in the flow stream (50).

10. A wearable cytometer according to claim 9, wherein the housing (12) includes:

a base (16);

a cover (18); and

a hinge (20) for securing the base (16) to the cover (18).

11. A wearable cytometer according to claim 9, wherein the removable cartridge (14) and the housing (12) are self aligned using one or more registration pins (28).

12. A wearable cytometer according to claim 9, wherein the removable cartridge (14) includes:

a fluid receiver (32) for receiving the sample fluid; and

at least one reservoir (64) for storing one or more supporting fluids.

13. A wearable cytometer according to claim 9, wherein the removable cartridge (14) includes a waste reservoir (52) downstream of the flow stream (50) for receiving and storing the fluid of the flow stream (50).

14. A wearable cytometer according to claim 12, wherein the sample fluid is a blood sample, and the one or more supporting fluids include lyse and sheath.

15. A wearable cytometer according to claim 14, wherein the removable cartridge (14) dilutes the blood sample, performs red cell lysing, and performs hydrodynamic focusing for flow and core stream formation.

16. A wearable cytometer according to claim 9, wherein the pressure applying means includes:

a pressure source for generating an input pressure;

a first pressure chamber (70) for receiving the input pressure;

a second pressure chamber (72);

first valves means (74) between the first pressure chamber (70) and the second pressure chamber (72); and

second valve means (76) for relieving the pressure in the second pressure chamber (72).

17. A wearable cytometer according to claim 16, wherein the pressure source includes a movable plunger that moves into the first pressure chamber (70).

18. A wearable cytometer according to claim 17, wherein the movable plunger is adapted to be manually depressed.

19. A wearable cytometer according to claim 16, wherein the first valve means (74) comprises a plurality of microvalves.

20. A wearable cytometer according to claim 19, wherein the plurality of microvalves are electrostatically actuated.

21. A wearable cytometer according to claim 20, wherein selected microvalves or groups of microvalves are individually addressable.

22. A wearable cytometer according to claim 16, wherein the second valve means (76) comprises a plurality of microvalves.

23. A wearable cytometer according to claim 16, wherein the replaceable cartridge (14) includes:

a downstream fluid stream in fluid communication with the second pressure chamber (72);

a flow sensor (80) for measuring the fluid flow in the downstream fluid stream; and

feedback means (40) coupled to the flow sensor (80) and to the first valve means (74) and the second valve means (76) for opening the first valve means (74) when the fluid flow in the downstream fluid stream drops below a first predetermined value and for opening the second valve means (76) when the fluid flow in the downstream fluid stream increases above a second predetermined value.

24. A wearable cytometer according to claim 23, wherein the flow sensor (80) is a thermal anemometer type flow sensor.

25. A wearable cytometer according to claim 24, wherein the thermal anemometer type flow sensor is a microbridge flow sensor.

26. A wearable cytometer according to claim 9, wherein the flow stream (50) has a length along a central axis (202) of flow and a width perpendicular to the central axis (202) of flow.

27. A wearable cytometer according to claim 26, wherein the light providing means (22) includes two or more spaced light sources (208, 210) positioned laterally at different distances from the central axis (202) of flow of the flow stream (50).

28. A wearable cytometer according to claim 27, wherein the two or more spaced light sources (208, 210) are positioned along a light source axis (200) that is rotated relative to the central axis (202) of flow of the flow stream (50).

29. A wearable cytometer according to claim 28, wherein the two or more spaced light sources (208, 210) provide substantially constant light intensity across the flow stream (50).

30. A wearable cytometer according to claim 29, wherein the two or more spaced light sources (208, 210) are part of an array of VCSEL devices.

31. A wearable cytometer according to claim 30, wherein the VCSEL devices operate in the red spectrum.

32. A wearable cytometer according to claim 30, wherein each of the two or more spaced light sources (208, 210) have a corresponding lens (220).

33. A wearable cytometer according to claim 32, wherein each lens (220) is an integrated micro lens.

34. A wearable cytometer according to claim 32, wherein selected lenses (220) focus the light at a central plane that includes the central axis (202) of the flow stream (50).

35. A wearable cytometer according to claim 34, wherein the light that is focused at the central plane is used for detecting the small angle scattering (SALS) produced by the selected particles in the flow stream (50).

36. A wearable cytometer according to claim 34, wherein the light that is focused at the central plane is used for determining the speed of selected particles in the flow stream (50).

37. A wearable cytometer according to claim 36, wherein the light that is focused at the central plane is used for determining the size of selected particles in the flow stream (50).

38. A wearable cytometer according to claim 32, wherein selected lenses (362) provide substantially collimated light through the flow stream (50).

39. A wearable cytometer according to claim 38, wherein the substantially collimated light is used for detecting the forward angle scattering (FALS) produced by the selected particles in the flow stream (50).

40. A wearable cytometer according to claim 9, wherein the selected particles include white blood cells.

41. A wearable cytometer according to claim 40, wherein the white blood cells include neutrophils and/or lymphocytes white blood cells.

42. A wearable cytometer according to claim 9, wherein the light providing means (22) includes a first set of light sources (302) positioned along a light source axis (200) that is rotated relative to the central axis (202) of flow of the flow stream (50), light from the first set of light sources is focused at a central plane that includes the central axis of the flow stream (50).

43. A wearable cytometer according to claim 42, wherein the light receiving means (24) includes a first set of light detectors (308) for receiving light from the first set of light sources (302) after the light passes through the flow stream (50), each of the first set of light detectors (308) having a corresponding lens (306) that focuses the light substantially on the corresponding light detector (308).

44. A wearable cytometer according to claim 43, wherein the first set of light sources (302) and the first set of light detectors (308) are used to detect the alignment of the flow of selected particles relative to the width of the flow stream (50).

45. A wearable cytometer according to claim 44, wherein the processing means (40) is in communication with the pressure applying means, and adjusts the controlled pressure applied by the pressure applying means to the one or more pressure receiving ports (34) of the removable cartridge (14) so that the alignment of

the selected particles in the core stream (160) is substantially in the middle of the flow stream (50).

46. A wearable cytometer according to claim 42, wherein the first set of light sources (302) and the first set of light detectors (308) are used to detect the velocity of selected particles in the flow stream (50).

47. A wearable cytometer according to claim 42, wherein the first set of light sources (302) and the first set of light detectors (308) are used to detect the size of selected particles in the flow stream (50).

48. A wearable cytometer according to claim 42, wherein the light providing means (22) includes a second set of light sources (320) positioned along a light source axis that is rotated relative to the central axis (202) of flow of the flow stream (50), each of the second set of light sources (320) having a corresponding lens (322) that focuses the light at a central plane that includes the central axis (202) of the flow stream (50).

49. A wearable cytometer according to claim 48, wherein the light receiving means (24) includes a second set of light detectors, each of the second set of light detectors having a detector region (328) laterally spaced from an in-line position of the corresponding light source (320).

50. An wearable cytometer according to claim 49, wherein each of the second set of light detectors has at least two detector regions (328, 330) for each of the second set of light sources (320), one (320) positioned in one direction relative to an in-line position of the corresponding light source (320) and another (330) positioned in another direction relative to the in-line position of the corresponding light source (320), each of the second set of light detectors having a corresponding lens (324) that focuses the light substantially on the corresponding light detector.

51. A wearable cytometer according to claim 50, wherein the second set of light detectors (328, 330) are used to detect the small angle scattering (SALS) produced by the selected particles in the flow stream (50).

52. A wearable cytometer according to claim 48, wherein the light providing means (22) includes a third set of light sources (360) positioned along a light source axis that is rotated relative to the central axis (202) of flow of the flow stream (50), each of the third set of light sources having a corresponding lens (362) that provides substantially collimated light through the flow stream (50).

53. A wearable cytometer according to claim 52, wherein the light receiving means (24) includes a third set of light detectors (368) positioned in line with the third set of light sources (360), each of the third set of light detectors having a corresponding lens (364) that focuses the substantially collimated light substantially on the corresponding light detector (368).

54. A wearable cytometer according to claim 53, wherein each of the third set of light detectors (368) are annular in shape.

55. A wearable cytometer according to claim 54, wherein the third set of light sources (360) and the third set of annular light detectors (368) are used to detect the forward angle scattering (FALS) produced by the selected particles in the flow stream (50).

56. A method for analyzing selected particles in a fluid stream, the method comprising:

manually generating a non-precise pressure;

generating a controlled pressure from the manually generated non-precise pressure;

providing force to a fluid stream using the controlled pressure; and
analyzing the selected particles in the fluid stream.

57. A method according to claim 56, wherein the analyzing step includes determining the alignment of the selected particles in the fluid stream.

58. A method according to claim 56, wherein the analyzing step includes determining the small angle scattering (SALS) produced by the selected particles in the flow stream (50).

59. A method according to claim 56, wherein the analyzing step includes determining the forward angle scattering (FALS) produced by the selected particles in the flow stream (50).

60. A method according to claim 56, wherein the manually generating step includes manually generating two or more non-precise pressures.

61. A method according to claim 60, wherein the generating step includes generating a controlled pressure from each of the two or more manually generated non-precise pressures.

62 A method according to claim 61, wherein each of the controlled pressures provide a force to a different fluid reservoir (62), wherein each fluid reservoir includes a different fluid.

63. A method according to claim 62, wherein the force that is applied to each fluid reservoir (62) provides a different velocity to each fluid.

64. A method according to claim 63, further comprising performing hydrodynamic focusing using each fluid to form the fluid stream.

65. A method according to claim 64, wherein the analyzing step includes determining the alignment of the selected particles in the fluid stream.

66. A method according to claim 65, further comprising adjusting each of the controlled pressures to provide a desired alignment of the selected particles in the fluid stream.

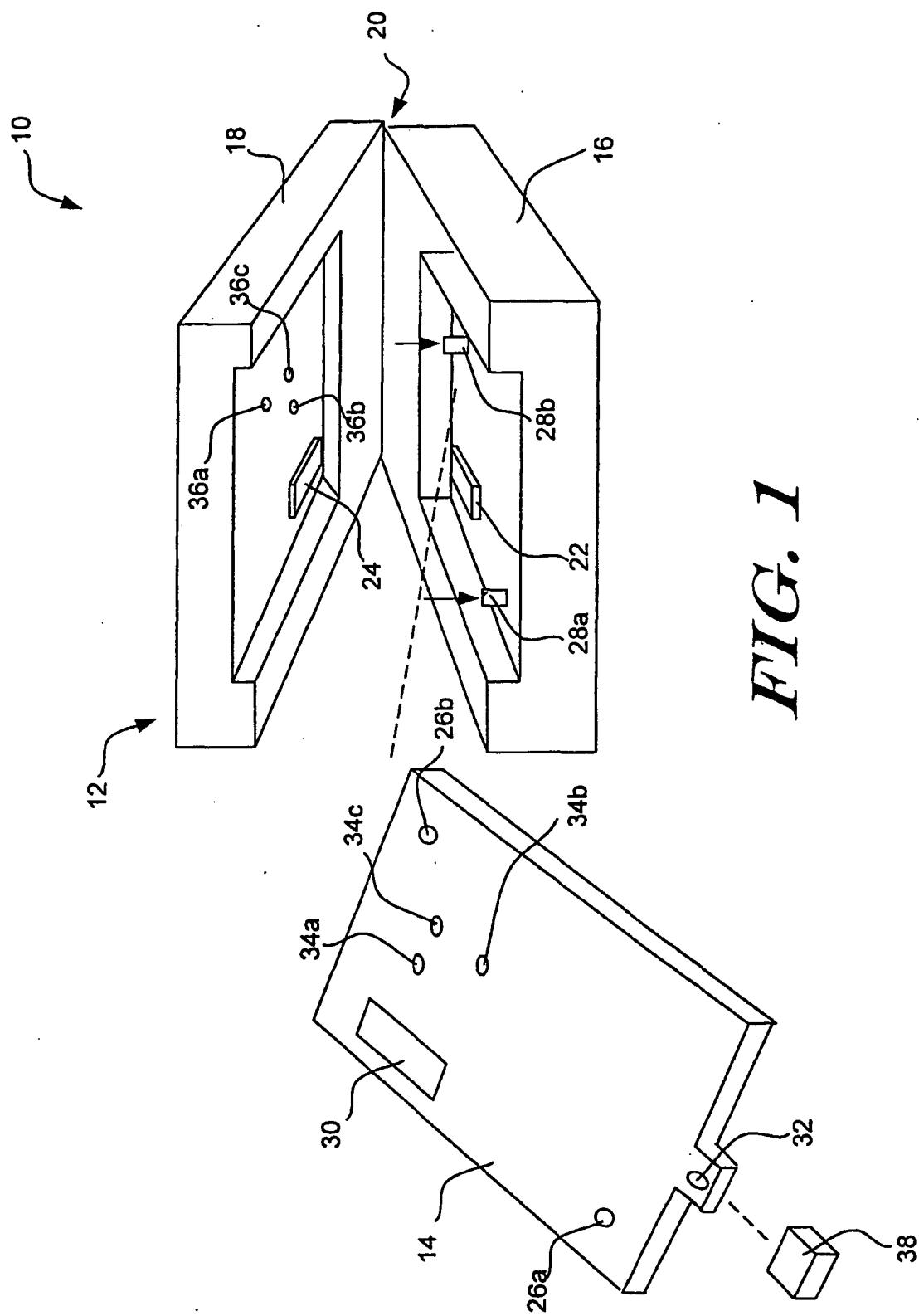


FIG. 1

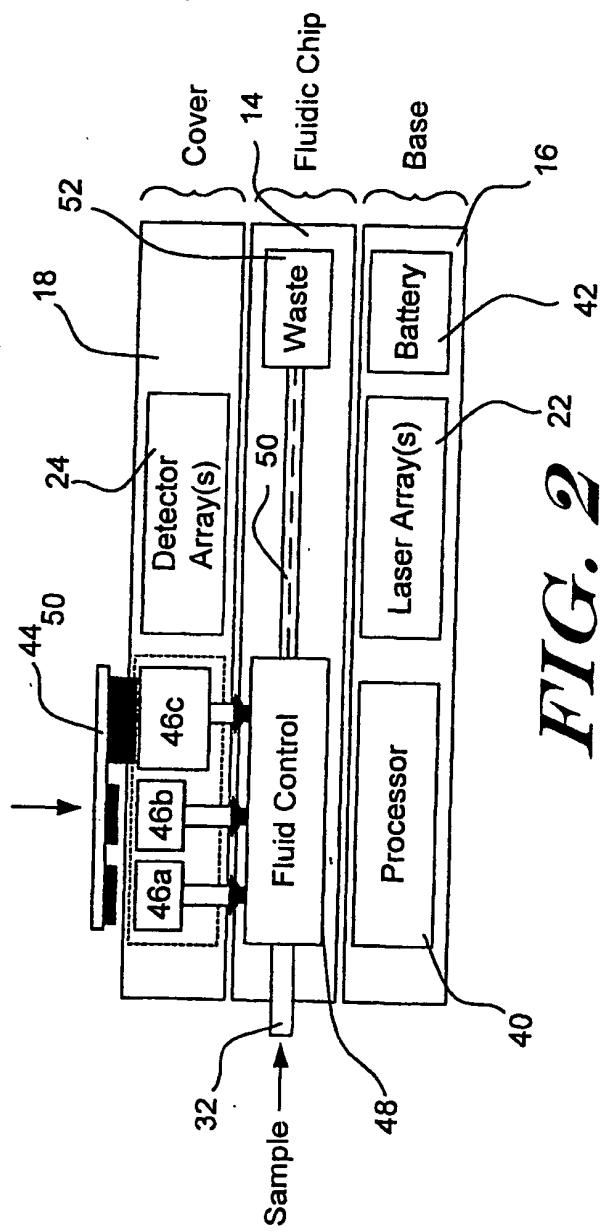
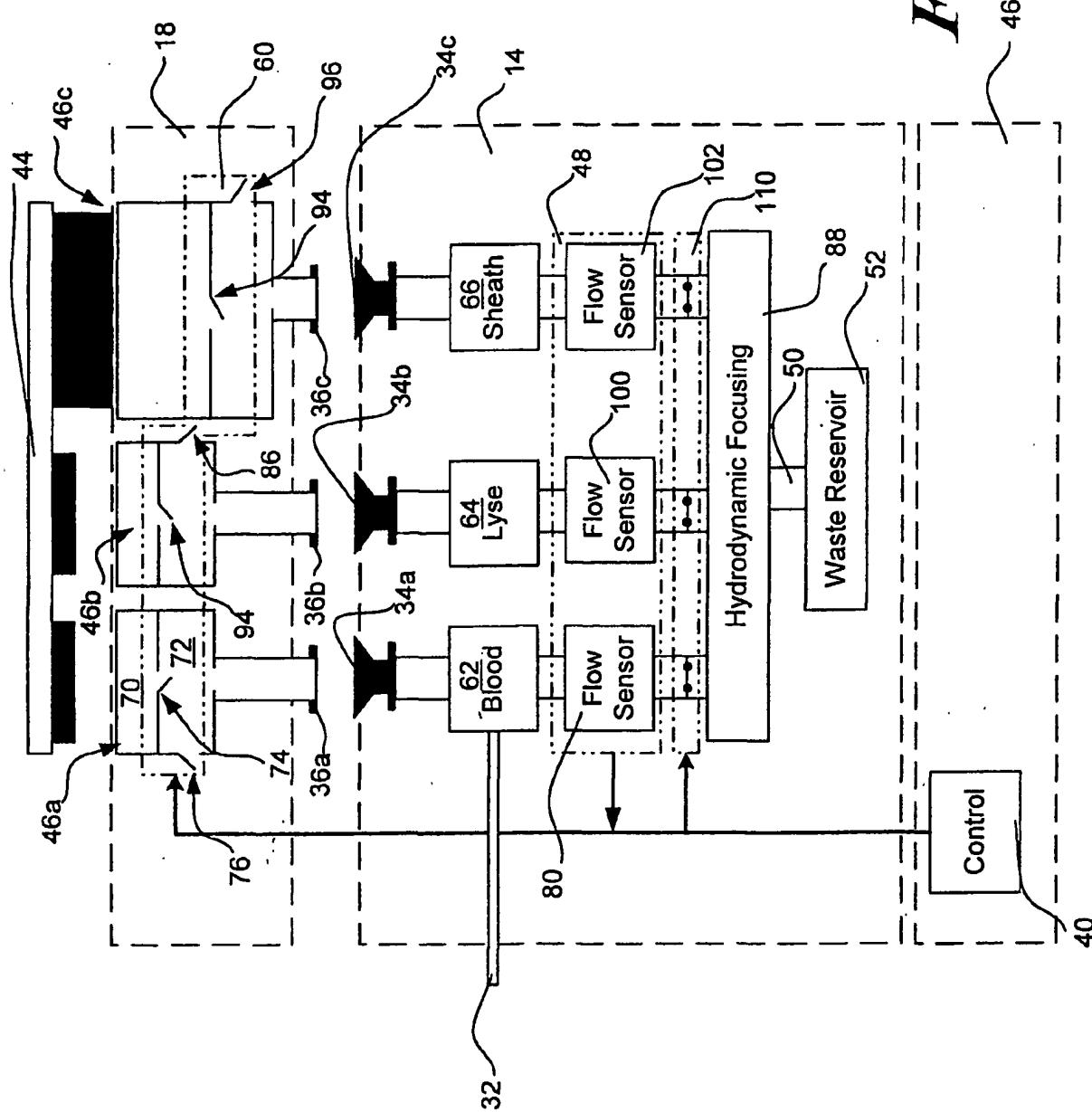


FIG. 2

FIG. 3



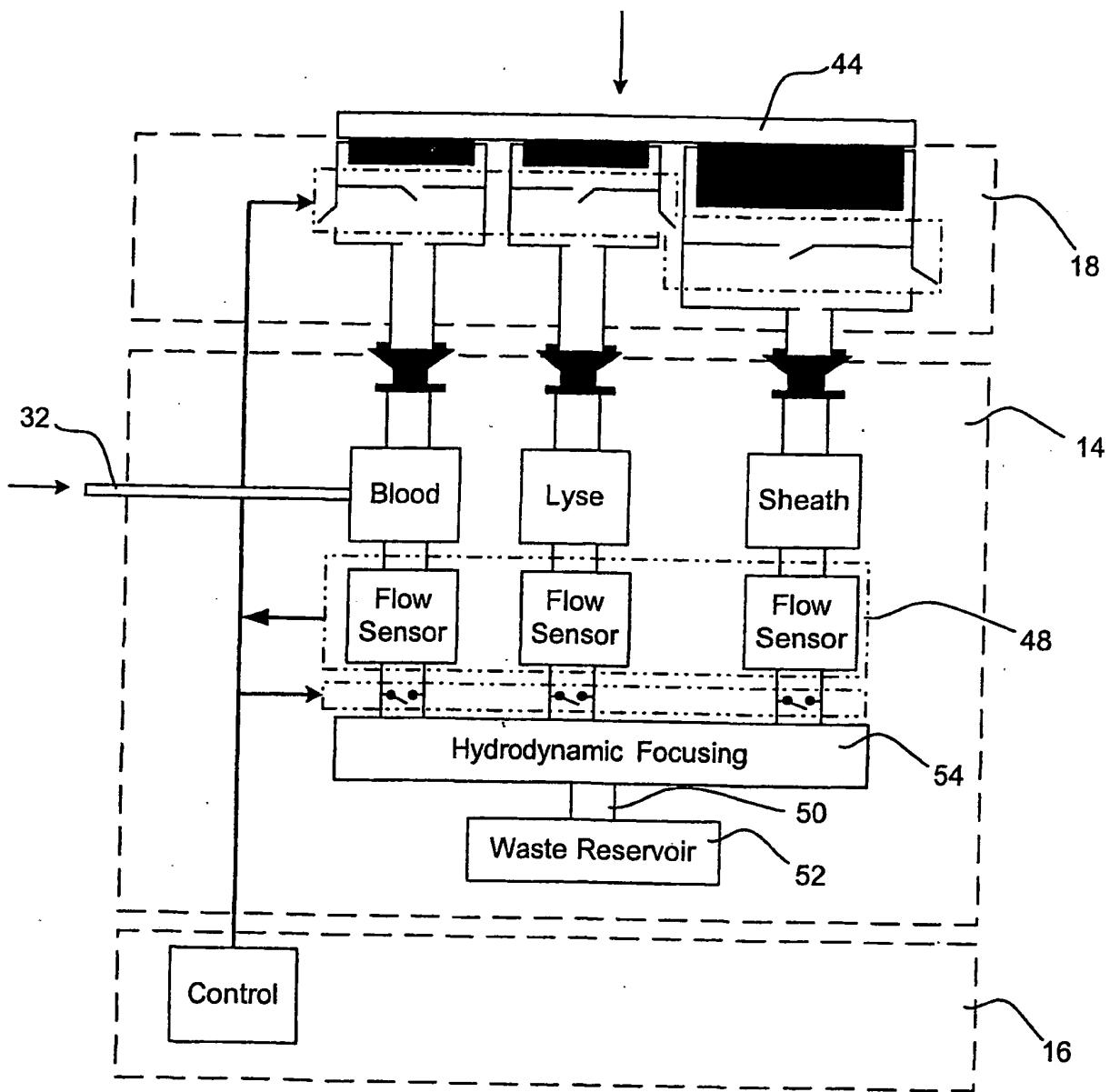


FIG. 4

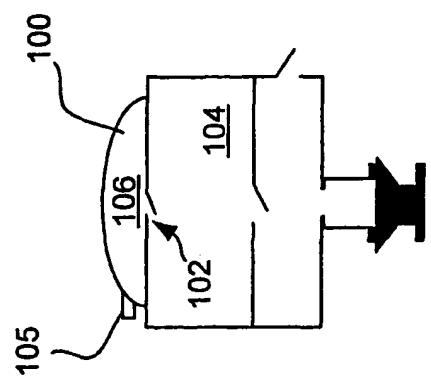


FIG. 5

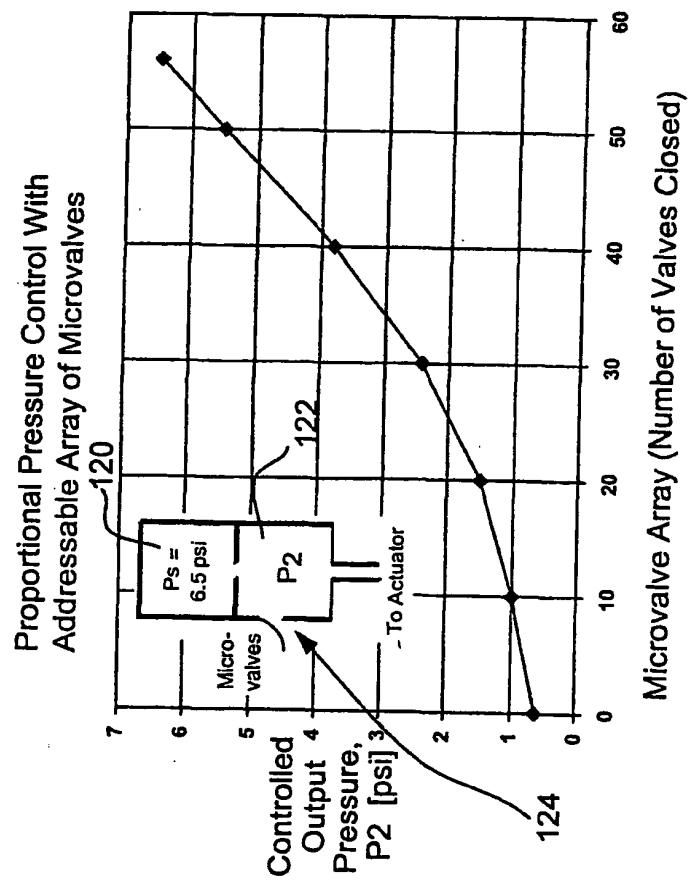


FIG. 6

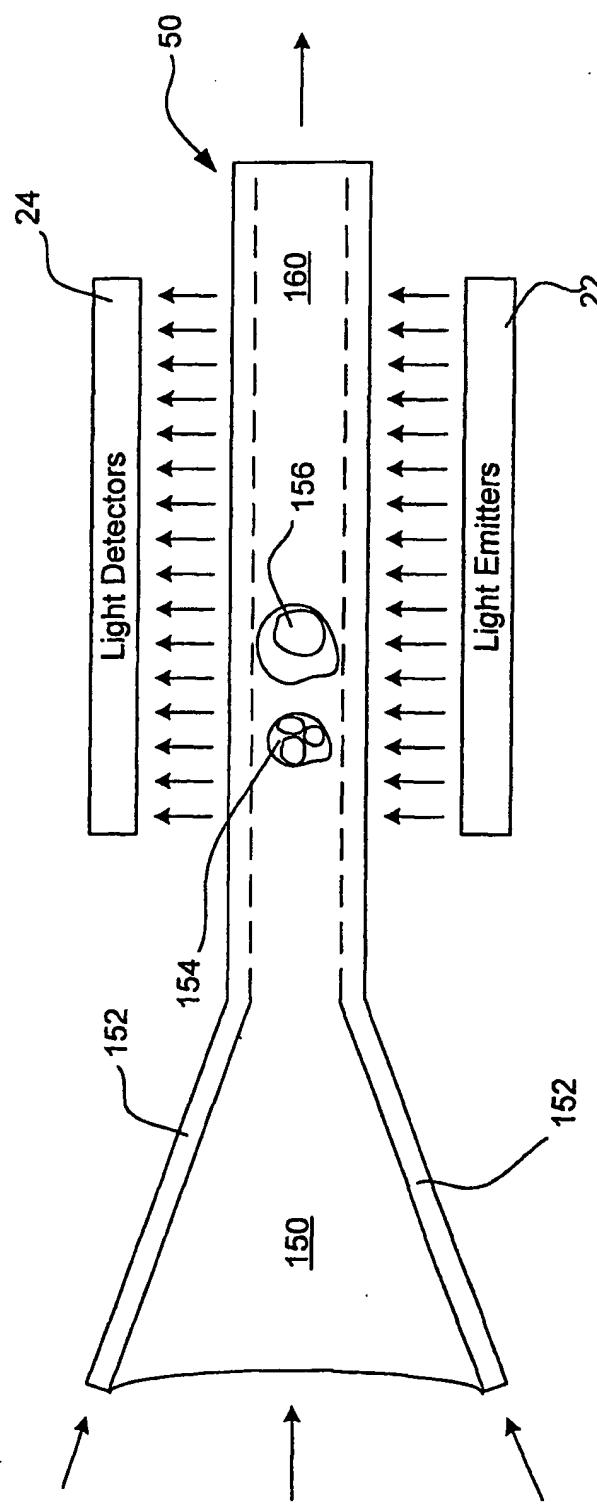
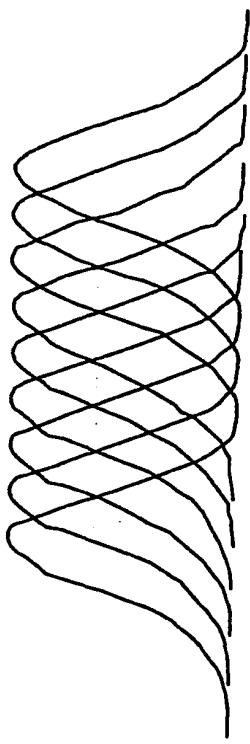


FIG. 7



+ + + + + + +

FIG. 9

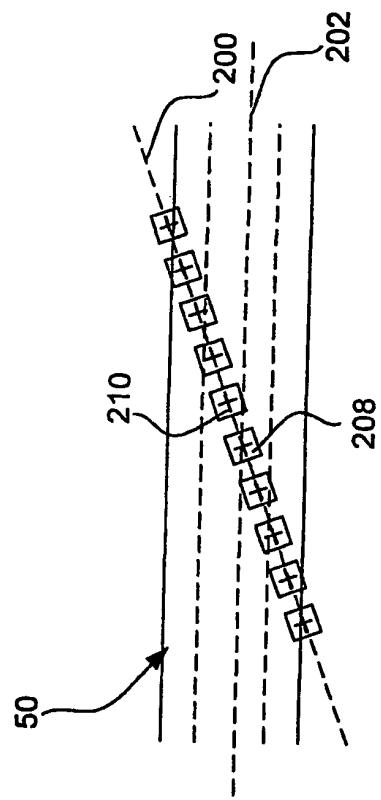


FIG. 8

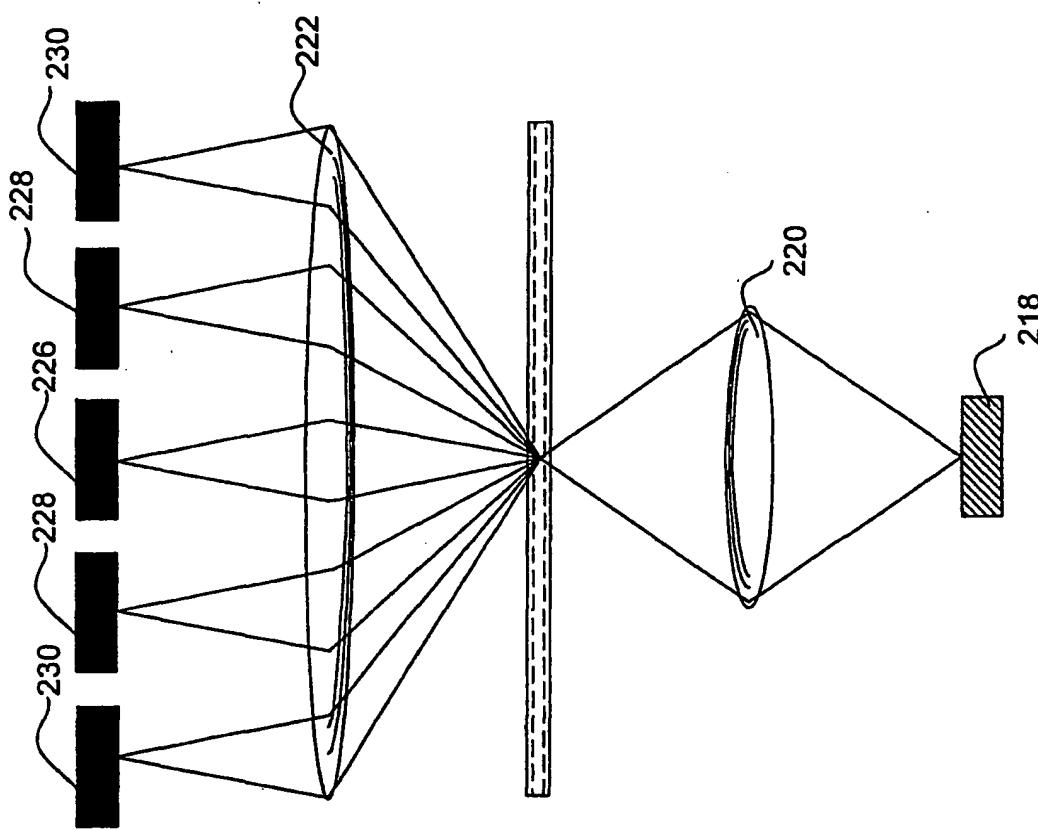


FIG. 10

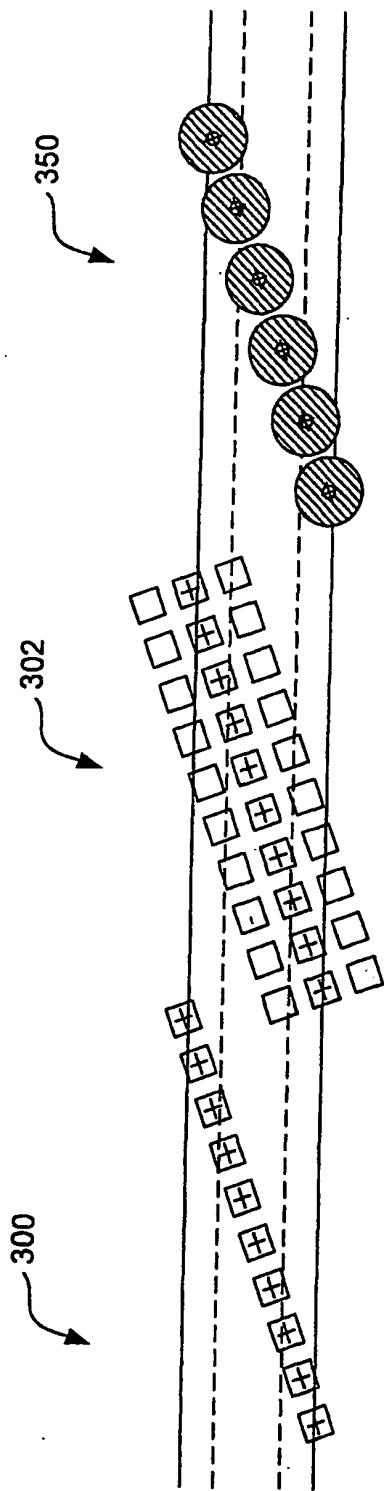
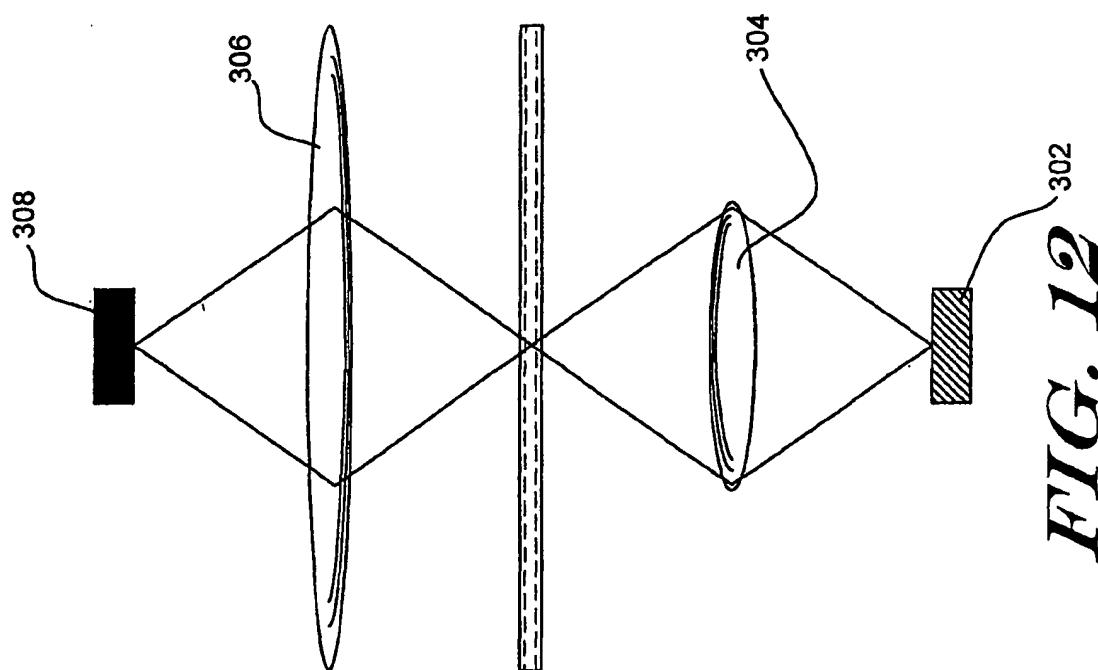
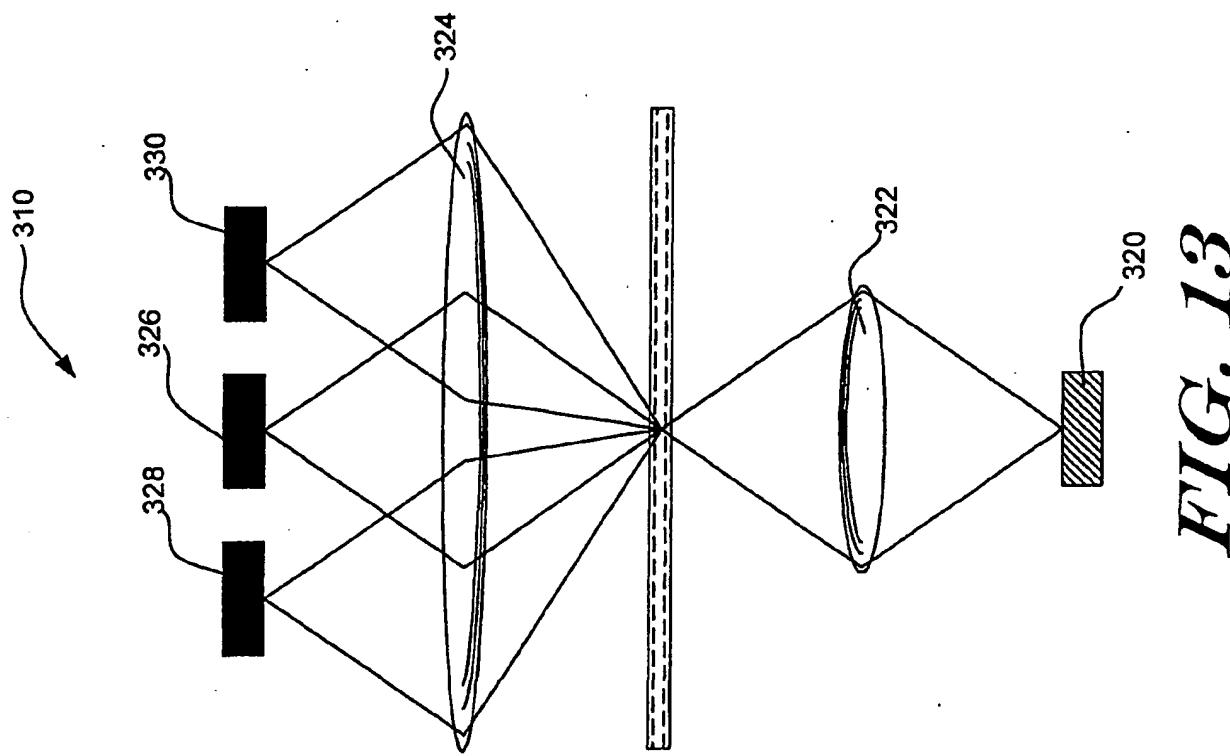


FIG. 11



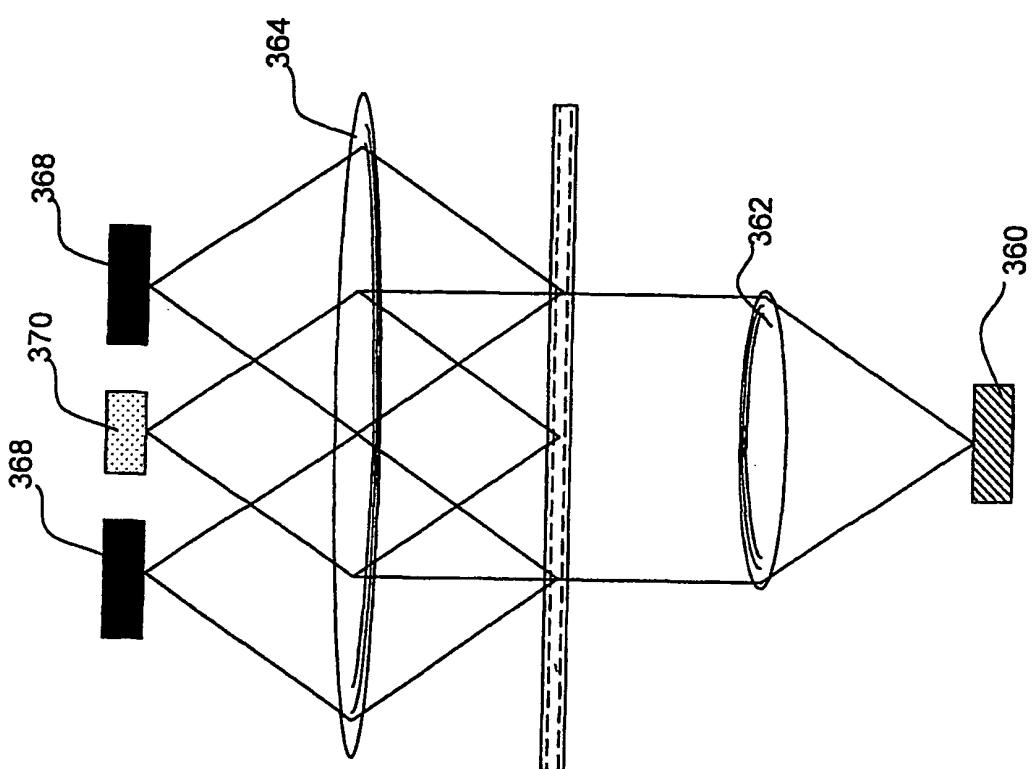


FIG. 14

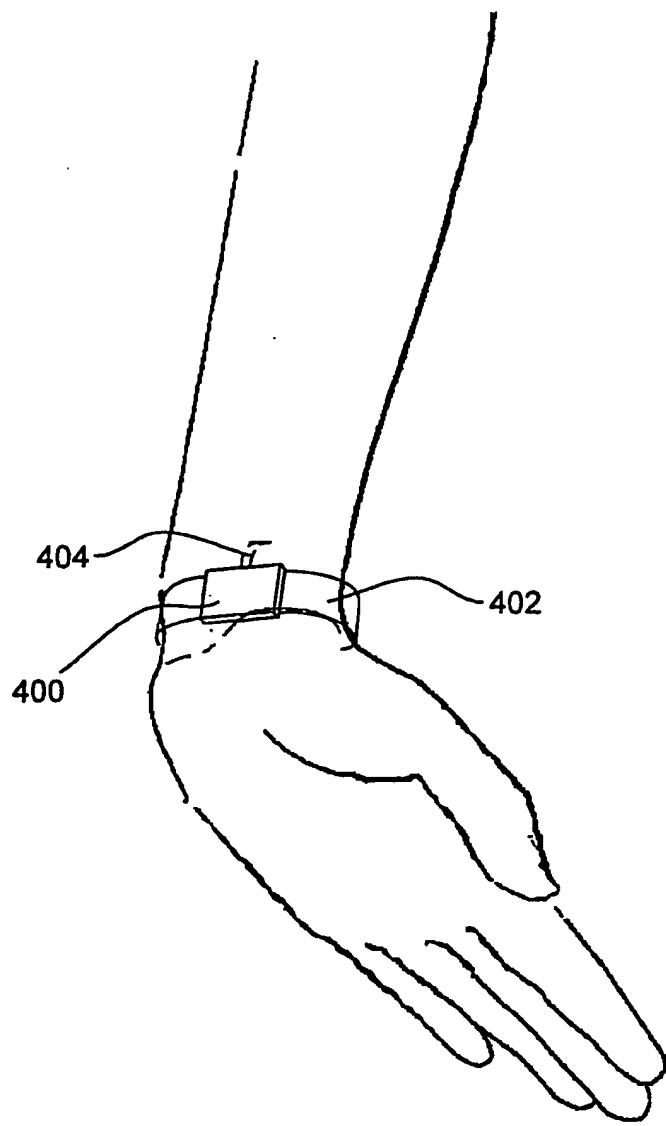


FIG. 15

